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Comparison of Habitat and Water Quality in the Blue River's South and Salem Forks in Southern Indiana to Support Watershed Management

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Table of Contents

List of Tables

List of Figures

Acronyms and Abbreviations

ANC.......................acid neutralizing capacity

BMP.......................best management practice

DO..........................dissolved oxygen

EPA........................United States Environmental Protection Agency

EPT/C.....................ratio of Ephemeroptera, Plecoptera, & Trichoptera to Chironomidae

FBI..........................Family Biotic Index

GIS..........................geographic information system

mIBI........................Macroinvertebrate Index of Biological Integrity

NaOH......................sodium hydroxide

PRS..........................phosphorus removal structure

SDI..........................Shannon Diversity Index

SpC..........................specific conductance

TNC.........................The Nature Conservancy

WMP.......................watershed management plan

WWTP.....................wastewater treatment plant

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Abstract

A stream's habitat and water quality are heavily influenced by land use and geology within its watershed. Pollutants and sediment loading from watershed drainage can make streams less habitable for certain species, reducing biodiversity. Watershed management strategies, such as the promotion of best management practices in agriculture, can help to combat stream degradation from watershed inputs. The upper Blue River in southern Indiana is a biodiversity hotspot but is experiencing degradation and biodiversity loss due to watershed inputs. This is exemplified by the disappearance of the eastern hellbender salamander, an indicator species, from this area. Fine sediment loading is particularly harmful to the hellbender because it causes embeddedness, decreasing habitat space within the substrate. Two watersheds of the upper Blue River, the Salem and South watersheds, differ significantly in land use and bedrock geology and have different watershed management strategies. The Salem watershed has higher percentages of urban and agricultural land, has predominantly limestone bedrock, and has a watershed management plan in place. Conversely, the South fork has a higher percentage of forested land and siltstone bedrock and does not have a watershed management plan in place. To determine how these watershed factors impact stream quality, five sites in the Salem fork and five sites in the South fork were tested for water chemistry, macroinvertebrate assemblage, and sediment distribution in the summer of 2017. Results suggested that high levels of phosphorus and fine sediment adversely affected macroinvertebrate ecology in the upper Blue River. Developed land provided significant phosphorus inputs, while agriculture and siltstone bedrock were the main sources of sediment loading. The South fork had better ecological conditions than the Salem fork and was less impaired by phosphorus. These results will be used by The Nature Conservancy, an organization that protects ecologically valuable areas, to aid in developing watershed management strategies and furthering conservation within the upper Blue River watersheds.

Introduction

Watersheds supply chemical and sediment inputs to streams through runoff and erosion (House et. al. 1993, Mallin et al. 2008). The types of land use and geology within a watershed significantly impact the chemical and biological composition of streams. Anthropogenic land uses, namely urbanization and agriculture, increase pollutant and sediment loading, which decrease water quality, habitat quality, and biodiversity (House et al. 1993, Carpenter et al.1998). The prevalence of impervious surfaces in urban areas increase stormwater runoff into streams, exacerbating urban water pollution and increasing streambank erosion and sedimentation (House et al. 1993, Mallin et al. 2008, Violin et al. 2011). Although lacking impervious surfaces, agriculture is often associated with excess nutrient loading and decreased riparian zones, which are natural buffers to erosion and pollution (Peterjohn and Correll 1984, Carpenter et al. 1998). Conversely, forested areas within watersheds, especially forested riparian zones, can act as drainage systems and buffers by processing nutrients, preventing pollutant runoff, and reducing flooding and erosion (Peterjohn and Correll 1984, Mayer 2005, Copler 2017).

Nutrients, particularly nitrogen and phosphorus, are necessary for stream productivity and under natural conditions, generally limit growth of primary producers, such as plants and algae (Dodds et al. 2002, Spahr et al. 2010). However, streams are considered polluted when anthropogenic nutrient loading occurs at too high a rate (Wang 2006, EPA 2008, IDEM 2013). Excess nitrogen and phosphorus can result in overgrowth of algae in streams (Dodds et al. 2002, Frankforter et al. 2009). As algae die, processes of decomposition increase overall respiration within the stream which can result in declining dissolved oxygen (DO) (Dodds et al. 2002). Aquatic animals rely on DO for survival; therefore, significant decreases in DO can result in the decline of species (Strayer et al. 1997, Smith et al. 1999). Even small changes in dissolved oxygen can impact macroinvertebrate communities, which are particularly susceptible to changes in water quality (Wang et al. 2006).

Shifts in macroinvertebrate communities are generally one of the first signals of changes in water quality and habitat quality within streams (Lenat 1988). Macroinvertebrates have a large presence within the hyporheic zone of streams, which is defined by White (1993) as the area of interstitial spaces within the substrate, separating the stream from the groundwater zone below. A significant proportion of stream respiration occurs within the hyporheic zone due to decomposition, so DO in the hyporheic zone is more dependent on respiration that it is in the surface water of a stream (Battin et al. 2003). Therefore, DO is more rapidly depleted within stream substrate than in surface water (Battin et al. 2003, White). This means that nutrient loading that causes higher respiration can have a substantial impact on the habitat viability for organisms living within the hyporheic zone (Battin et al. 2003). Macroinvertebrate taxa have different ecological requirements, and some taxa tend to tolerate pollutants and DO reductions better than others. As pollution increases and habitat quality worsens, relatively intolerant taxa, such as Ephemeroptera, Plecoptera, and Trichoptera, typically decline, allowing tolerant species to become more dominant (Lenat 1983). Nutrient pollution can actually benefit some tolerant macroinvertebrates that feed on algae, such as certain chironomids, by increasing food availability (Lenat 1988).

The reliance of macroinvertebrates on benthic and hyporheic zones within streams also makes them susceptible to embeddedness, or excess fine sediment deposition in a stream's substrate (Strayer et al. 1997, Sullivan and Watzin 2008). Embeddedness reduces habitat space by filling in pore spaces in substrate with fine sediment (Levine 2013). Streambank erosion is a significant cause of the sediment loading that causes embeddedness (Fox et. al 2016). Human activities such as riparian zone destruction and urbanization increase erosion by causing bank instability and increased flooding (Simon and Collision 2002, Violin et al. 2011). High levels of embeddedness negatively affect biotic communities by reducing the interstitial spaces in the substrate that they or their prey rely on for shelter (Sullivan and Watzin 2008).

Bedrock within a watershed affects stream chemistry and substrate composition and can be a natural cause of embeddedness (Neff and Jackson 2011, Olson 2012). Due to its chemical and physical weathering properties, sedimentary bedrock correlates with higher alkalinity, conductivity, and turbidity (Peters 1984, Neff and Jackson 2011). Certain ions, like calcium, are necessary for the survival of organisms and are higher in streams with sedimentary bedrock, especially limestone (Jeziorski et al. 2008, Neff and Jackson 2011, Olson 2012). While sedimentary rocks like shale, siltstone, and sandstone tend to erode into fine particles and contribute to embeddedness (Olson 2012), limestone dissolves into ions as it erodes and does not have an equivalent impact on embeddedness (Peters 1984).

The eastern hellbender salamander (*Cryptobranchus alleganiensis alleganiensis*) is experiencing significant declines in abundance due in part to embeddedness (Nickerson et al. 2003, Burgmeier et al. 2011, Levine 2013, Rossell et al. 2013). Because of these declines, the eastern hellbender is listed as a Federal Species of Concern (USFWS 2016). Hellbenders, like many amphibians, are sensitive to poor water quality and require cool, flowing water (Ultsch and Duke 1990, Nickerson et al. 2003, Burgmeier et al. 2011, Rossell et al. 2013). They are most successful in streams with low embeddedness. Adults show preference for gravel substrate and rely on large rock slabs for daytime shelters and nests (Nickerson et al. 2003, Burgmeier et al. 2011). Larval hellbenders are not well studied but have been found in interstitial spaces in sediment in the hyporheic zone and beneath small rocks (Nickerson et al. 2003). Hellbenders feed primarily on crayfish and other macroinvertebrates (Nickerson et al. 2003, Burgmeier et al. 2011). Embeddedness, pollution, and lack of prey are thought to be the main drivers of hellbender decline, so studying these factors may assist in conservation and reestablishment of the species (Burgmeier et al. 2011).

The United States Environmental Protection Agency (EPA) presents a potential solution to the nutrient, sediment, and other pollutant loading that affects aquatic species like hellbenders by encouraging watershed-scale stream management through Watershed Management Plans (WMPs) (EPA 2008). WMPs include evaluation of current stream conditions, goals for future pollutant reductions and habitat improvements, and management strategies that are needed in order to obtain these goals (EPA 2008). These management strategies, which are methods to reduce stream pollution from urban and agricultural runoff, are collectively known as Best Management Practices (BMPs) (EPA 1993, EPA 2008). The success of WMPs depends on the amount of involvement from stakeholders, and results are not immediate as stream conditions may take months to decades to show significant improvements (Meals et al. 2010).

The watersheds of two main forks of the upper Blue River in southern Indiana (Figure 1), the Salem fork and the South fork, differ in land use, bedrock geology, and watershed management. The Blue River is a tributary to the Ohio River that has high overall biodiversity due to protected forests that cover much of its lower watershed, preserving habitat and water quality (TNC 2017). The Nature Conservancy, a nonprofit organization that protects ecologically valuable areas, considers this watershed to be a conservation priority (TNC 2017). Nutrient loading poses a substantial threat to the Blue River. Carlson Mazur et al. (2016) found a significant positive relationship between agriculture and nitrate loading in the Blue River and a detrimental effect of agriculture on macroinvertebrate metrics. Forested land, on the other hand, was associated with decreased nitrate and improved macroinvertebrate metrics, suggesting that forested land can prevent or lessen stream degradation (Carlson Mazur et al. 2016). Previous studies have concluded that the river tends to have inferior water quality and habitat quality in the upper watershed because it contains more agricultural and urban land and is less forested (Summers 2007, Carlson Mazur et al. 2016).

Degradation in the Blue River's upper watershed has caused declines in eastern hellbenders, which are native to the river (TNC 2017). The extant population is found mostly or entirely in the lower watershed (Nick Burgmeier, personal communication, 22 March 2017). Hellbender surveys in the Blue River have indicated significant declines in population density, lack of immature individuals and little evidence of reproduction, and a gender ratio that is skewed toward males (Burgmeier 2011). Unger et al. (2013) estimated that without management, hellbenders would vanish from the Blue River within 25 years. The extant hellbenders in the Blue River exist mostly in the lower watershed, and reintroduction efforts have been focused in this region (Burgmeier 2011, Kraus 2017). Researchers at Purdue University have considered reintroducing hellbenders into the South fork of the upper Blue River in addition to the release locations in the southern part of the river (Nick Burgmeier, personal communication, 22 March 2017). However, prior observations of embeddedness and poor riparian buffers led to the conclusion that the South fork is an inadequate site for reintroduction (Nick Burgmeier, personal communication, 22 March 2017). Results of this study will be used to assess the ability of each fork to meet conditions necessary for hellbender habitat.

Of the two forks in the upper Blue River, the Salem fork is managed by means of a WMP (IDEM 2013). This plan, the Mill Creek-Blue River Watershed Management Plan, was introduced in 2013 to encourage land management strategies that will help meet water quality goals in the watershed (IDEM 2013). The WMP encourages farmers to employ BMPs that include techniques such as no-till agriculture and planting cover crops along with restoration methods like bank stabilization and tree planting along riparian zones (IDEM 2013). The South Fork watershed does not have a management plan established.

To support watershed management efforts in the Salem and South forks of the Blue River, habitat quality, water quality, and sedimentation were examined. Objectives of the study were to (1) compare water chemistry, macroinvertebrate assemblages, and substrate sediment distributions in the South and Salem forks and (2) determine effects of watershed characteristics such as land use and bedrock geology on conditions within the stream, specifically water chemistry, macroinvertebrates, and sediment distribution. Based on its lack of a WMP, the South fork was expected to have a stronger association between agricultural land use and stream degradation than the Salem fork. An association between agricultural and urban land uses and stream degradation was hypothesized due to the relationship between these land uses and nutrient and sediment loading. Nutrient loading and sediment loading were both expected to deteriorate ecological quality. Recommendations are included for future watershed management options that would reduce human impacts to the Blue River and increase viability of returning hellbenders to the stream.

Methods

Site Description

Five sites from the South Fork and five sites from the Salem Fork were selected for analysis between July 7 and August 3 of 2017 (Figure 2). Sites were chosen at relatively evenly spaced locations along the forks where landowner permission was granted. Seven sites, five from the Salem Fork and two from the South Fork, were included in previous studies in the Blue River (Summers et al. 2007, Mazur et al. 2016), and three were added in this study (TNC17, TNC19, and TNC20).

Comparatively, the Salem fork that runs through the city of Salem is more urban, and the South fork is more rural (Figure 3). The underlying geology in each watershed differs (Figure 4). Both watersheds contain predominately limestone bedrock, but the percentage of limestone is higher in the Salem watershed than in the South watershed, and the South watershed contains substantially more siltstone (Figure 4).

Site Setup and Water Chemistry Sampling

At each site, a 50-meter reach that included riffle, run, and pool habitats was measured. Weather conditions, time of sampling, and ordinal date, which is a specific numerical value assigned to each day of the year, were recorded. Water chemistry sampling, macroinvertebrate sampling, discharge measurements, and streambed particle-size sampling were performed within the reach. Water chemistry was sampled upstream of the riffle at each site. A YSI Pro-DSS meter was used to test temperature, pH, specific conductance (SpC), DO, and turbidity. Acid neutralizing capacity (ANC) was tested onsite using a Hach alkalinity kit.

Water samples were collected at each site for nitrate and total phosphorus tests. The water samples were preserved by adding 5 drops of sulfuric acid onsite and later freezing them. Prior to testing, the samples were thawed and neutralized with NaOH. Total phosphorus was measured using a HACH TNT 843 total phosphorous spectrophotometer, and nitrate was measured using a Nitra Ver X Reagent spectrophotometer test. Both tests were conducted at Manchester University in North Manchester, Indiana.

Macroinvertebrate Sampling, Identification, and Calculations

Macroinvertebrates were collected using a modified protocol from Barbour et al. (2015). First, a kick screen was used for one minute to sample macroinvertebrates in one square meter of the riffle. Then, multi-habitat sampling was performed by distributing thirty jabs with a D-net approximately proportionally in each habitat present (Sobat 2010). Collected material was sifted, moved to a collection plate, and picked through for organisms for a combined total of 15 minutes (five minutes for the riffle sample, 10 minutes for the multi-habitat sample). The collected macroinvertebrates were preserved in 95% ethanol and identified to family later in the laboratory under a dissecting microscope. Organisms in the Chironomidae family were identified to tribe, as required by various indices of biodiversity.

The Macroinvertebrate Index of Biodiversity (mIBI); Shannon Diversity Index (SDI); Family Biotic Index (FBI); total abundance; taxa richness; and ratio of Ephemeroptera, Plecoptera, and Trichoptera to Chironomidae (EPT/C) were calculated for each site. FBI, which is also known as the Hilsenhoff Biotic Index, measures the mean family tolerance of the sample. EPT/C determines how the abundance of pollution-intolerant species compares to the abundance of the relatively pollution-tolerant Chironomidae family (Barbour et. al. 2015). For every metric except FBI, higher values reflect better ecological conditions. Since FBI is based on tolerance, the opposite is true.

Discharge Measurement

Discharge (m^3/s) was measured along a transect at the widest portion of a run within the reach. A Hach FH950 portable flow meter and depth pole were used to measure flow velocity (m/s) and depth (m) at several points along the transect for calculation of discharge at each site. A measurement point was taken at any significant depth change along the transect. A range of nine to 18 data points were collected. Due to instrument failure at the TNC20 (Big Springs Rd) site, velocity was measured manually by measuring transit time for an object to float 0.25 meters downstream. Discharge was calculated for all sites from width, depth, and velocity measurements following Rantz et al. (1982).

Sediment Distribution Sampling and Calculation

To assess the levels of embeddedness in the Salem and South forks, a streambed particle-size count was performed at each site a using a United States Geological Survey standard gravelometer. Five pebbles were sampled along 21 transects in the reach for a total sample size of 105 pebbles. The pebbles were selected using a modified step-toe procedure in which the collector walks in a predetermined pattern from bank to bank along the reach and selects the particle with each step (Bunte and Abt 2001). For this study, particles were chosen at 0% (left bank), 25%, 50%, 75%, and 100% (right bank) of the wetted width of the stream. The step-toe procedure was modified by submerging a meterstick at each approximated location without intentional bias, tracing a finger down the meterstick, and retrieving the first particle that was felt. The smallest gravelometer sieve size that each pebble fit through was recorded (Bunte and Abt 2001). Sizes were recorded in the phi (φ) scale, which is described by Bunte and Abt (2001) as the standard scale for sediment distributions (Table 1). Clay- and silt-sized particles ($\varphi = 8.0$ and 4.0, respectively) were sized based on collector estimation rather than by sieve.

Substrate particle sizes were analyzed by creating particle-size frequency distributions for each site. Distribution graphs were compared visually, and particle size percentiles were compared between sites (Bunte & Abt 2001). Normality of each distribution was determined visually . Fine sediment loading was analyzed by calculating the percentage of the sample size that was classified as fine sediment. Fine sediment was defined as any particle less than 2mm (greater than -1 in φ units). This was calculated for the total sample, for the particles on the interior portion of the stream transects (25%, 50%, and 75% of transect width) and for the particles along the stream bank (0% and 100% of transect width).

Geographic Information System (GIS) Analysis

ArcMAP 10.4 (ESRI 2016) was used to determine land use and bedrock percentages within each site's individual upstream watershed. Previous watershed delineations from the seven sites in the Blue River study by Carlson Mazur et al. (2016) were used. For the three new sites included in this study, watersheds were delineated by hand using in GIS using digital elevation model raster data (USGS 2013) and streamlines from the National Hydrography Dataset (USGS 2008). Bedrock geology and land use layers from Indiana MAP (IGS 1987, 2011) were then clipped to each watershed, and percentage of watershed occupied was calculated for various land use categories and bedrock types.

Statistical Analysis

After each site was sampled, the data were analyzed to compare the streams and test for relationships between variables. Initial data analysis included two-tailed t-tests, calculated in Microsoft Excel 2016, to test for significant differences between mean water quality measurements, macroinvertebrate calculations, median particle size, and watershed bedrock and land use percentages in each fork. Pearson correlations were conducted in SPSS Statistics 25.0 (IBM 2017) for all variables to identify significant correlations to guide further analysis.

Multiple linear regressions were performed in SPSS to test the effects of various watershed variables on water chemistry metrics, macroinvertebrate metrics, and sediment distribution (

Table 2). Effects of interaction variables were considered in multiple linear regressions. Models were chosen based on R^2 values and significance at the $\alpha = 0.05$ level of confidence.

Results

Salem and South Fork Comparison

Differences in mean water chemistry values were apparent between the Salem and South forks, although none were significantly different in t-tests (Table 3). The Salem fork had higher mean SpC, ANC, DO, nitrate, and total phosphorus than the South fork (Table 3). Average temperature and turbidity were higher in the South fork (Table 3). Mean pH values were similar between watersheds (Table 3).

Macroinvertebrate metrics also differed between watersheds. Mean FBI was significantly lower in the South fork than the Salem fork (Table 3), indicating greater presence of organisms intolerant of pollution. The South fork also had a higher mean EPT/C, mIBI score, SDI, taxa richness, and total abundance, but these differences were not significant (Table 3).

Sediment distributions were similar between both forks, and no significant differences were detected through t-tests (Table 3). The South fork had higher mean sediment sizes for D16 (16^{th} percentile) and D25 (25^{th} percentile) (Table 4), which suggests lower proportions of fine sediment, and lower percent interior fine sediment (Table 3), but significant differences between watersheds were not detected (Table 3, Table 4). Based on visual analysis of histograms, all sites had high fine sediment loading (Figure 5, Figure 6).

GIS analysis showed several significant differences in land use and bedrock between watersheds. The Salem fork sites' watersheds had significantly higher mean percentages of limestone bedrock, while mean siltstone percent was higher in the South fork (Table 3). Mean percent developed land, open space, and total agriculture were greater in the Salem fork (Table 3). Within total agricultural land, the Salem fork watersheds also had significantly higher mean percentages of both hay and pasture land, and cultivated crop land in their watersheds (Table 3). Mean percent forested land was greater in the South fork (Table 3). Average watershed area in each fork was nearly equivalent (Table 3).

Phosphorus Impacts and Sources

Total phosphorus was significantly associated with a decline in macroinvertebrate community metrics, as shown by the negative relationship between total phosphorus and both EPT/C and mIBI in multiple linear regressions (Table 5). In one of the two regressions with EPT/C that contained phosphorus, this effect was only seen when watershed was taken into account, indicating a greater negative impact of total phosphorus on EPT/C in the Salem fork than the South fork. This was also true between phosphorus and mIBI (Table 5).

Simple linear regressions showed that urbanization, or the sum of developed land and open space percentages, in the Salem fork had a significant positive relationship with phosphorus

(Figure 7). Forested land in the Salem fork showed a negative relationship with phosphorus; as the percent of forested land in the watershed increased, total phosphorus in the stream decreased (Figure 7). TNC13 (Salem WWTP) was excluded as an outlier in these regressions due to its high nitrate and total phosphorus levels resulting from sewage effluent. In the South fork, the same effects of urbanization and forested land on phosphorus were not evident in simple linear regressions (Figure 7).

In a multiple linear regression including both forks, percent developed land and forested land both significantly increased phosphorus, although the effect of developed land was over 50 times greater than that of forested land (Table 6). The interaction term of developed land multiplied by forested land in this regression had a negative effect on phosphorus (Table 6).

pH and DO Impacts and Sources

pH and DO both had apparent beneficial effects on macroinvertebrate metrics (Table 5). As with total phosphorus, these effects were seen with EPT/C and mIBI (Table 5). In the same regression in which phosphorus displayed a negative impact on EPT/C, pH positively affected EPT/C. In a separate regression, DO positively affected EPT/C, more so in the South fork than the Salem fork (Table 5). DO also was related to an increase in mIBI in two separate multiple linear regressions, one where watershed was considered and one where it was not (Table 5).

Nitrate and DO were positively related to pH (Table 6). In a multiple linear regression, increases in DO and nitrate both were related to an increase in pH (more basic), mediated by the interaction term of DO and nitrate (Table 6). In simple linear regressions comparing the effects of DO on pH between the Salem and South forks, the positive effect of DO on pH was only significant in the Salem fork (Figure 8). While percent limestone was expected to increase pH, no effects were detected between limestone and pH (Figure 8).

DO likely had multiple influencing factors. Temperature had a strong negative correlation with DO, indicating that cold water holds more oxygen (Table 8). Percentages of forested land, cultivated crops, and limestone within a watershed were also associated with increased DO (Table 6). The effects of forested land and cultivated crops were similar, but each of their coefficients was over four times greater than that of limestone (Table 6).

Indirect Impact of Nitrate and Sources

No significant effects of nitrate on macroinvertebrate metrics were detected. However, nitrate increased pH (Table 6) and was very strongly associated with open space (Figure 9), both of which improved EPT/C (Table 5). Nitrate may have come from several different sources. Simple linear regressions showed that as percent of developed land, open space, and cultivated crops within a watershed increased, nitrate levels increased (Figure 9). As percent forested land increased, nitrate decreased.

Fine Sediment Impacts and Sources

Based on visual analysis of histograms (Figure 5, Figure 6), overall sediment distributions did not show normality. This was because sediment distributions were skewed to varying degrees toward fine sediment at every site (Figure 5, Figure 6). Every distribution except TNC11 (Horner's Chapel Rd) appeared more normal when fine sediment was excluded (Figure 5, Figure 6). Bank samples were skewed much more heavily toward fine sediment than were interior samples (Table 7).

The percent of fine sediment in the interior of the stream detrimentally impacted macroinvertebrate metrics (Table 5). Percent interior fine sediment decreased mIBI and SDI, and increased FBI, all of which suggest deteriorated macroinvertebrate communities (Table 5). Total percent fine sediment and percent bank fine sediment did not have any detected effects on macroinvertebrate metrics.

Because only the interior fine sediment showed effects on macroinvertebrates, the potential sources of interior fine sediment were analyzed. Percent interior fine sediment increased as percent agriculture and siltstone increased in the watershed (

Table 9). Higher percent bank fine sediment also increased percent interior fine sediment (Table 9). Forested land was related to decreased percent interior fine sediment (

Table 9).

Effects of Sampling Date

The 27-day range within which sites were sampled had an effect on macroinvertebrate diversity and turbidity. The ordinal sampling date had significant positive correlations with taxa richness and with SDI (Table 8). This effect was also seen in a multiple linear regression that showed sampling date positively affecting SDI (Table 5). In this regression, the effect of the other independent variable, percent interior fine sediment, was only seen when ordinal date was taken into account. Ordinal date also had a very strong positive correlation with turbidity (Table 8).

Other Significant Findings

Other significant relationships established by this study included an effect of SpC on macroinvertebrates and an effect of turbidity on total phosphorus. SpC displayed a negative effect on macroinvertebrates in one regression (Table 5). In a regression that included DO, phosphorus, and percent interior fines, higher SpC was associated with decreased mIBI (Table 5). Phosphorus had an effect on turbidity that was opposite of expectations. Total phosphorus had a significant negative correlation with turbidity (Table 8).

Numerous other regressions and correlations were tested but are not shown, either because they were not significant or because a stronger explanatory model was chosen. Full results are summarized in a conceptual effects model (Figure 10).

Discussion

Watershed features are major determinants of water and habitat quality within streams, and in this study the Salem and South forks of southern Indiana's upper Blue River were no exception. Agriculture and urbanization within the Salem and South watersheds determined nutrient levels and, along with watershed bedrock type, affected sedimentation. Together, nutrients and sedimentation altered macroinvertebrate communities and perhaps have affected the overall biota within the upper Blue River.

Nutrient Loading and Primary Production

Nutrient inputs stimulate growth of primary producers and are essential to overall stream productivity (Dodds et al. 2002, Spahr et al. 2010). However, anthropogenic nutrient inputs pollute waterways by causing overgrowth of algae, which can reduce light penetration, increase turbidity, and cause hypoxia (Mallin et al. 2008, Frankforter et al. 2009, Spahr et al. 2010). Two main nutrients, total phosphorus and nitrate, impacted macroinvertebrate habitats in the upper Blue River differently. Based on negative impacts of total phosphorus on EPT/C and mIBI, phosphorus inputs harmed macroinvertebrate communities. Nitrate inputs had little detected effect on macroinvertebrate metrics and may have indirectly benefitted communities.

Phosphorus is found in fertilizers and in organic waste from sewage and animal manure (Mallin et al. 2008, Fox et al. 2016). Because phosphorus clings to soil particles, erosion can be a major driver of phosphorus inputs (Mallin et al. 2008, Fox et al. 2016). However, total phosphorus was negatively associated with turbidity and lacked a relationship with streambed fine sediment. While a negative relationship between turbidity and phosphorus may seem contradictory based on known associations between suspended sediment and phosphorus (Fox et al. 2016), it can be explained by the relationship between phosphorus and urban land uses. In this study, the main sources of phosphorus were open space and developed land, which will be referred to collectively as “urbanization.” Urbanization tended to decrease turbidity in the Salem and South forks, suggesting that there may have been reduced erosion in urban areas. While impervious surfaces found in urban areas can lead to increased erosion after storm events (Mallin et al. 2008), this either did not affect erosion or did not have as extreme an effect on erosion as agriculture, one of the main sources of fine sediment loading in this study. Whether due to the lack of agricultural soil disturbance in developed areas or to impervious surfaces acting as a cap over soils, urbanization in the Salem and South forks was associated with less erosion. Few major rain events occurred during the sampling period, so this result may have differed had major flooding occurred.

The absence of an association between phosphorus and erosion of sediment in urban areas suggests two more likely sources of phosphorus: fertilizer runoff and sewage inputs and leaks. Sewage may have been a main contributor since sewer systems are more specific to developed land, whereas fertilizers are associated with both developed and crop land. Fertilizer runoff could also have played a role being that impervious surfaces can exacerbate runoff by not allowing natural filtration of pollutants like phosphorus from soil and vegetation. While organic forms of phosphorus generally lack mobility across landscapes, phosphorus within fertilizer is often highly water soluble and therefore much more mobile (Fox et al. 2016).

The effect of developed land on total phosphorus was substantial in the Salem fork that contained more developed land than the South fork. Total phosphorus inputs in the South fork did not show the same dependence on developed land. This difference is likely because the mean percent developed land in the South fork watersheds was only about a third of that in the Salem fork watersheds and was spaced out more sporadically. Likewise, EPT/C was not as affected by total phosphorus in the South fork as it was in the Salem fork. Ecological research suggests that aquatic ecosystems can have alternative stable states, with regard to nutrient and other pollutant levels, and that catastrophic state shifts can occur when levels reach a certain tipping point (Scheffer et al. 2001). Based on this theory, total phosphorus may elicit a stronger ecological response in the upper Blue River after a critical level is reached, at which point it will continue to have detrimental effects unless phosphorus loading substantially declines (Scheffer et al. 2001). It is possible that the Salem fork had surpassed a critical point and undergone a catastrophic shift while the South fork had not. If this was the case, phosphorus loading may need to decrease to levels below the critical point in order to return to the pre-disturbance stable state (Scheffer et al. 2001). Due to the small sample size and lack of prior data on total phosphorus, however, more research would be needed to confirm this.

Stream nitrate inputs come from fertilizers and organic matter within runoff (Mallin et al. 2008). Based on significant regressions, cultivated crop land, open space, and developed land likely all added nitrate to the upper Blue River via fertilizer runoff. Impervious surfaces within urban areas exacerbate nitrate loading by increasing runoff and flooding (Mallin et al. 2008, Violin et al. 2011). While results did not reveal any direct relationships between nitrate and macroinvertebrates, other regressions signified that nitrate could have had an effect. For example, open space significantly contributed nitrate, and open space also positively affected EPT/C in one regression model. Nitrate also had a positive relationship with pH, which in turn had a positive relationship with EPT/C. The beneficial impacts of nitrate may reflect the intermediate disturbance hypothesis, in which moderate levels of disturbance such as nutrient loading increase diversity because neither intolerant nor tolerant species achieve dominance (Townsend et al. 1997). Higher primary productivity resulting from greater nutrient availability likely aided some macroinvertebrates by increasing DO and food resources for herbivorous macroinvertebrates. This supports findings by Copler (2016) that showed a beneficial impact of nitrate on EPT/C in the Blue River.

Despite the abundance of limestone in the upper Blue River watershed, biological rather than geological factors determined pH, since pH was dependent on nitrate and DO levels in the stream. Increases in primary productivity from added nitrate likely increased photosynthesis, which raised pH by removing carbon dioxide from the water (Frankforter et al. 2009). Water chemistry was sampled during the daytime, ranging between 9:30 am and 2:30 pm, when photosynthesis was likely occurring at high rates in open stream areas (Frankforter et al. 2009). The positive effect of nitrate on pH suggests that nitrate loading was not having eutrophic effects, because during eutrophication, the impact of nitrate on pH may be reversed due to high respiration (Frankforter et al. 2009). However, the data suggest that the higher the nitrate level, the less DO affected pH. This is supported by the negative effect of the interaction term of nitrate and DO on pH. At a certain saturation point, nitrate likely no longer served to increase pH.

Based on the high nitrate and phosphorus levels at site TNC13, which was located at the Salem wastewater treatment plant, the treatment plant likely contributed substantially to both nutrients through wastewater effluent. Although samples were conducted upstream of a visible effluent pipe, total phosphorus was almost three times greater than that of any other site, and nitrate was more than double the next highest site. Both values well exceed the recommended values stated in the Mill Creek-Blue River Watershed Management Plan (IDEM 2013). TNC13 had the second lowest EPT/C ratio, a metric that is often negatively affected by organic pollution (IDNR 2013). While sampling at TNC13, four dead crayfish were found in the reach and no living crayfish were found. While this is anecdotal, it may suggest that conditions at TNC13 were toxic to some intolerant organisms since crayfish are generally considered to be moderately intolerant to pollution in Indiana (IDEM 2018).

Forested land seemed to reduce the impact of developed land use on phosphorus inputs into the stream. The negative impact of the interaction of developed and forested land on total phosphorus likely showed that the higher the percentage of forested land in a watershed, the less developed land effected phosphorus. Considered on its own, forested land had much more of an impact on total phosphorus in the Salem fork than in the South fork. Due to its ability to mitigate urban phosphorus inputs, this could just be a result of the significantly higher percent developed land in the Salem fork. Forested land also appeared to decrease nitrate levels. However, it is unclear whether this effect was a result of nutrient processing within forested areas or because crop land and urban land, the main nitrate sources, decreased as forested land increased. Followup analysis could help determine the effects of riparian versus inland forests and of other vegetated areas such as wetlands.

Sediment Loading

The sediment distributions in the Salem and South forks had disproportionately high levels of fine sediment loading. This supports previous observations of high embeddedness within the river (Burgmeier, personal communication, 22 March 2017). Based on the impacts of percent interior fine sediment on macroinvertebrate metrics, sediment loading poses a significant problem for ecological health in the upper Blue River. Since embeddedness causes habitat loss for species that reside in the interstitial spaces of stream substrate, high fine sediment loading in a stream's interior can result in species loss and community alterations (Strayer et al. 1997, Sullivan and Watzin 2008). Fine sediment also can contribute to turbidity, which blocks sunlight for macrophytes, further damaging habitat (Mallin et al. 2008). Turbidity did not have a detectable negative impact on organisms in this study, however.

Agricultural areas were a likely source of fine sediment in the stream. Deforested riparian zones and poor soil conservation measures in agricultural areas can result in bank erosion (Simon and Collision 2002). Bank incision and deforested riparian zones were observed at several sites. Since the percent of interior fine sediment was strongly associated with the percent of bank fine sediment at each site, bank erosion clearly contributed to fine sediment loading.

Siltstone was also a major contributor of fine sediment. As the percent of siltstone bedrock within the watershed increased, percent interior fine sediment increased. Siltstone erodes into fine sediment. Limestone, the other main bedrock type in the upper Blue River, can contribute fine sediment also but, due to its calcium carbonate composition, tends to dissolve as it erodes (Peters 1984). The majority of siltstone in the upper Blue River was in the South watershed. Therefore, a greater amount of fine sediment loading occurred as a result of siltstone in the South fork than it did in the Salem fork. The opposite was true of agriculture, which made up a much greater percent of total land use in the Salem fork than in the South fork. Therefore, agriculture was likely the main contributor of sediment in the Salem fork as opposed to siltstone.

TNC15 was treated as an outlier in sedimentation analysis because of the very high sediment loading due to factors unrelated to land use or geology. The discharge at TNC15 was nearly 0 m^3/s due to a debris dam. The lack of discharge caused a large pool to form that trapped sediment. This situation was unique to TNC15 and, therefore, was incomparable with sedimentation conditions at other sites. Since TNC15 has both high siltstone (54.5%) and high agriculture (52.8%), it could be a useful site for future research determining sources of fine sediment loading.

Forested land likely reduced the amount of fine sediment loading in the stream. Riparian forests help to stabilize banks with their root systems and reduce erosion into the stream (Simon and Collision 2002). Forests act as sponges within watersheds, absorbing water to reduce runoff into the stream that leads to flooding, a major cause of erosion (Dwyer et al. 1992).

Sediment sampling is prone to certain biases by the collector. The sampler tends to show bias against small particles because these are more difficult to be identified and retrieved by touch than larger particles (Bunte & Abt 2001). Therefore, the high prevalence of fines in the sample is more likely to be an underrepresentation rather than an overrepresentation. Some of the skewness, however, may be explained by the fact that most exposed bedrock was covered with a thin layer of fine sediment and was thus categorized as fine particles rather than bedrock based on sampling protocol that required the sampling of the first particle identified by touch. There also tends to be bias both for and against large particles (Bunte & Abt 2001). The meterstick used for sampling tended to slip off of larger surfaces, which may have introduced bias. The bias toward larger particles is inherent, since large particles take up more space and are statistically more likely to be randomly selected than small particles (Bunte and Abt 2001). It also should be emphasized that the percent fines calculation is a percentage of the sample size and is not related to substrate volume or mass, so it can only be considered in terms of sampling frequency.

Ecological Implications

Previous studies have shown that the upper Blue River is experiencing significantly more ecological deterioration than the downstream portion of the river (Summers et al. 2007, Carlson Mazur et al. 2016). Eastern hellbenders have been extirpated from this portion of the river (Burgmeier, personal communication, 22 March 2017). The results of this study may provide insights into why this is the case, where the issues are the worst, and what can be done to improve conditions.

Of the stream variables tested, total phosphorus and sediment loading seemed to have the most detrimental effects on macroinvertebrate communities, particularly in the Salem fork. Losses, and changes within macroinvertebrate communities affect organisms up the food chain who rely on these species for prey. For example, if the dead crayfish observed at TNC13 are representative of toxic conditions for all crayfish at that location, then hellbenders and other predators that feed on crayfish may be unable to persist at this site.

Since phosphorus did not have as strong an effect on macroinvertebrate biota in the South fork as it had in the Salem fork, the South fork may be better suited for predator species like the hellbender. However, levels of embeddedness in the South fork were comparable to the Salem fork, which was consistent with previous observations. As was noted by Burgmeier, the South fork may be too embedded for successful hellbender reintroduction in its current state (personal communication, 22 March 2017).

The main contributors of embeddedness in the Salem and South forks may be different, however, suggesting that embeddedness may not be as harmful to ecology in the South fork as it is in the Salem fork. This study suggested that sediment loading in the Salem fork was related largely to anthropogenic causes while natural siltstone bedrock formations may have been the main contributor in the South fork. Therefore, some level of embeddedness in the South fork may be natural and could have been present when hellbenders inhabited the fork. Unfortunately, no quantitative data could be found from previous studies to determine whether embeddedness has been stable or changing over time. A comparison study could be conducted between the South fork and other largely siltstone-bedded streams with variable degrees of anthropogenic land use to help determine whether the sedimentation levels are indeed representative of natural conditions.

Certain sample sites show that the South fork may be suitable for hellbender reintroduction. TNC11 (Horner's Chapel Rd), in particular, stood out as a potential reintroduction site. TNC11 had the lowest percent fines (interior) of all sampled sites (3.2%), displayed comparatively high ecological health based on macroinvertebrate metrics, and sustained a high number of crayfish (26), the preferred prey of adult hellbenders (Nickerson et al. 2003, Burgmeier et al. 2011). Several large limestone slabs that did not appear embedded were also noted at TNC11, which could provide potential shelters for a hellbender population (Nickerson et al. 2003, Burgmeier et al. 2011). TNC20 (Big Springs Rd) also had vegetated riparian zones, relatively low percent fines (interior) (12.7%), and high ecological health metrics. Based on water quality, the South fork may be more suitable than the Salem fork for reintroduction because it lacks a negative relationship between phosphorus and macroinvertebrate community health.

Effectiveness of WMP

This study was unable to determine the effectiveness of the Mill Creek-Blue River WMP in improving stream quality of the Salem fork. As a comparison study, the results were able to show that the Salem fork was more degraded than the South fork, but there was no way of concluding whether this represented a lack of effectiveness of the WMP. Data from this study could be compared via statistical analysis with data from past and future studies to help determine the WMP's effectiveness. Although land use is certainly causing degradation to the Salem fork, the WMP is relatively new and effects of watershed management take time. Suggested improvements and BMPs cannot be implemented all at once, and improvements to water and habitat quality tend to occur slowly (Meals et al. 2010).

Limitations of the Study

The results of this study are limited by the small sample size of ten sites. While several significant results were detected, any random chance events or undetected equipment malfunctions could have a strong influence on the data. The strong significance of several regression models within the study suggests relationships not due to chance, but further research with more sample sites could help support these findings. Additionally, while substantiated guesses could be made as to relationships specific to the Salem or South fork individually, finding significant regressions for an individual fork was nearly impossible due to the sample size of five sites.

Sampling dates had an unintended effect on two main metrics of the study, SDI and turbidity. SDI tended to increase over the sampling period, likely because of the strong correlation between sample date and taxa richness, which is used in calculating SDI. Based on this relationship, it is likely that, due to life cycle differences, certain species had not hatched or were not large enough to be seen easily when sampling during earlier dates and became more viable for sampling later in the summer.

Additionally, the most recent land use data available were used in this study but likely did not perfectly depict current land use during the time of sampling. Some changes have likely occurred to the landscape between 2011, the year the GIS land use layer used in this study was created, and the time of sampling in 2017.

Recommended Actions

The detrimental impact of total phosphorus on ecological health found by this study support efforts to reduce phosphorus loading, particularly from urban areas, which were the main sources of phosphorus loading. A synthetic approach to phosphorus removal is the construction of phosphorus removal structures (PRS), which contain materials that absorb phosphorus (Penn et al. 2017). Storm water and wastewater effluent pipes and other sites with high discharge rates into streams are good candidates for PRS placement to maximize efficiency and cost-effectiveness (Penn et al. 2017). Types of PSRs differ based on materials used, and Penn et al. (2017) details the efficiency of several types of commonly used PSRs. Since the Salem watershed's ecology was significantly impacted by phosphorus, PSRs could be constructed at sites in and near the city of Salem of heavy discharge into the stream, such as the Salem WWTP effluent pipe.

Wastewater effluent from the Salem WWTP contributed significant phosphorus and nitrate into the stream, which impaired the ecological health of the TNC13 site. The data suggest that improved nutrient removal at the WWTP would significantly benefit the ecology of the

Salem fork at this site and may be beneficial to the fork and Blue River as a whole. This study could not establish whether the nutrient loading at this site also impacted downstream sites, however, which may be because only two sites were downstream. Increased monitoring and nutrient-reduction measure could be taken to reduce the WWTP's impacts as a point-source of nutrient pollution. Along with the option of adding a PSR to remove phosphorus, infrastructural improvements could be considered. However, if such measures are not an option due to budget, space, or labor limitations, an EPA study showed that several optimization techniques can effectively reduce nutrient loading without major infrastructural changes (EPA 2015). Optimizations that reduce phosphorus include creating a zone within the secondary treatment process to add enhanced biological phosphorus removal using volatile fatty acids, which can be created by fermenting existing sludge, and adding metal salts to precipitate orthophosphate during tertiary treatment (EPA 2015). Depending on affordability, creation of an artificial wetland onsite and discharging into the wetland rather than directly to the stream can effectively use natural systems to remove nutrients without major infrastructural changes (EPA 2015). These and other adjustments, such as optimization of activated sludge and creation of nutrient-removing lagoons are detailed in EPA (2015).

The strong association between total phosphorus and urbanization in the Salem fork watershed suggests that nonpoint sources like fertilizer runoff over impervious surfaces and sewage and septic leaks likely contribute phosphorus, so measures should be taken to slow runoff rates and improve nutrient processing within the city of Salem. Options to help slow runoff include encouraging the use of permeable pavement and rainwater collection with rain barrels (Ahiablame et al. 2013). Permeable pavement allows precipitation to soak into the soil rather than rapidly running off into waterways (Ahiablame et al. 2013). To combat sewage inputs, regular maintenance of sewer lines and septic systems should also be encouraged for the city and for individuals. Natural options to reduce nutrient loading include restoration of riparian buffers along stream banks and creation of artificial wetlands in low-lying areas (Peterjohn and Correll 1984). The data strongly support riparian restoration near urban areas based on the finding that forested land reduces the effect of developed land on phosphorus loading.

Riparian restoration is also an important focus in agricultural areas because fine sediment loading, which was significantly linked to agriculture, had a negative impact on ecological health. Since increased forested land was associated with decreased fine sediment loading, reforesting riparian zones along streams and drainage ditches within agricultural areas would likely decrease sediment loading and consequently improve ecological health over time. Other methods of controlling sediment loading from agriculture, such as the use of cover crops and no-till methods should also be considered since these practices have been shown to decrease sediment and nutrient loading (EPA 1993, EPA 2008). Future research could look at where these and other BMPs are being used and whether there is an association between these practices and improved stream quality. This would also help to determine the effectiveness of the Mill Creek-Blue River WMP in the Salem fork.

Whereas active nutrient- and sediment-load reduction efforts are suggested for the Salem fork, the data suggest that the South fork is less degraded overall, is not ecologically impacted by phosphorus loading, and experiences fine sediment loading largely from siltstone rather than agriculture. While active load reductions may not be needed in the South fork, preservation and monitoring are essential to ensuring the protection of existent forests in the South fork watershed and managing increases in urbanization and agriculture if they occur. This will be particularly important if hellbenders are reintroduced to the stream. Based on the findings of low anthropogenic impacts on nutrient and fine sediment levels in the South fork, certain sites in the fork could be reconsidered for hellbender recolonization. More research, including analysis of substrate pollutant concentrations and specific comparisons of South fork conditions with conditions in locations where hellbenders currently persist, could be useful in determining whether the South fork meets habitat requirements for hellbender reintroduction. Since the South fork substrate likely has a natural tendency toward a high percentage of fine sediment from siltstone, any added sediment loading from bank erosion and agriculture could potentially make the stream uninhabitable for organisms like hellbenders. Therefore, protecting bank stability and preventing erosion from agriculture are essential to preserving ecological conditions within the South fork.

Conclusion

The results of this study indicated that land use and geology had significant impacts on the conditions of the South and Salem forks in the upper Blue River watershed. Phosphorus inputs from urban areas impaired macroinvertebrate communities, especially in the Salem fork, whose watershed contained a significantly greater percentage of urban land than the South fork's watershed. Fine sediment loading, which resulted from agricultural land use and siltstone bedrock geology, also harmed macroinvertebrate assemblages. Agriculture was likely the main source of sediment to the Salem fork, whereas siltstone was likely the main source in the South fork. Overall, the South fork displayed higher ecological health and less impacts from anthropogenic land use than the Salem fork.

Based on these findings, future watershed management would benefit from a focus on total phosphorus and fine sediment load reductions, particularly in the Salem fork, to help improve ecological health. A strong emphasis should be placed on reforestation as a watershed management strategy, considering the impact of forested land on reducing the effects of urban phosphorus loading and the negative effect of forested land on percent fine sediment in the interior of the stream. The findings of this study emphasize the significance of both anthropogenic and natural watershed features in determining ecological quality of streams and support the use of watershed-level management to improve stream conditions.

Table 1. Phi (φ) scale for streambed sediment sizes. To convert between mm- and φ-values, the equation $\varphi_i = -3.3219 \log(D_i)$ is used, where φ_i is the particle diameter on the φ scale and D_i is the diameter in mm.

size (mm)	**size (φ)**
0.0039	8.0
0.063	4.0
2	-1.0
2.8	-1.5
4	-2.0
5.6	-2.5
8	-3.0
11	-3.5
16	-4.0
22.6	-4.5
32	-5.0
45	-5.5
64	-6.0
90	-6.5
128	-7.0
180	-7.5
> 180	<-7.5

Table 2. Variables tested during analysis of Salem and South fork water chemistry, macroinvertebrate, and sediment data collected in summer 2017.

Category	**Dependent Variable**	**Independent Variables**
Water Chemistry	temperature, SpC, ANC, DO, nitrate, total phosphorus, turbidity	bedrock geology, land use, discharge, percent fines
Macroinvertebrate Assemblage	mIBI, taxa richness, total abundance, EPT/C, FBI	water chemistry metrics, bedrock geology, discharge, land use, percent fines
Sediment Distribution	Percent fines (total, interior, and banks)	Bedrock geology, land use, discharge

Table 3. T-tests for significant differences in water chemistry, macroinvertebrate, streambed, and watershed metrics between the Salem and South watershed sites.

	Mean		Variance				
Parameter	**Salem**	**South**	**Salem**	**South**	**t-stat**	**df**	**P-value**
Water Chemistry							
Temperature (˚C)	22.94	24.08	0.29	1.73	-1.794	8	0.111
SpC (µS/cm)	456.50	274.44	30227.29	6622.59	2.121	8	0.067
ANC (mg/L)	135.4	99.4	1582.3	3951.8	1.082	8	0.311
DO (mg/L)	7.80	6.22	1.25	1.64	2.083	8	0.071
Nitrate (mg/L)	3.44	1.00	5.825	0.39	2.188	8	0.060
Total Phosphorus (mg/L)	0.4026	0.2442	0.1188	0.0033	1.014	8	0.340
pH	7.89	7.85	0.01	0.18	0.225	8	0.828
Turbidity (FNU)	4.72	5.78	30.37	3.49	-0.407	8	0.694
Macroinvertebrates							
mIBI score	30	32	20	8	-0.845	8	0.423
Taxa richness	16.8	17.8	10.7	8.7	-0.508	8	0.625
Abundance	74.2	76.0	172.7	441.5	-0.162	8	0.875
EPT/C	3.13	7.18	5.23	14.30	-2.053	8	0.074
FBI	4.41	3.67	0.16	0.19	2.813	8	0.023*
Shannon Diversity Index (SDI)	2.41	2.46	0.06	0.06	-0.344	8	0.739
Streambed Features							
D50 (φ units)	-1.1	-3.4	26.3	0.925	0.986	8	0.353
Percent fines (total)†	30.48	26.48	62.28	44.17	0.827	7	0.435
Percent fines (interior) †	18.65	16.19	103.09	83.65	0.382	7	0.713
Percent fines (banks) †	48.21	42.86	24.09	73.70	1.103	7	0.307
Watershed Features							
Discharge (cms)	0.35	0.16	0.13	0.01	1.137	8	0.288
Siltstone (%)	20.92	68.68	371.53	471.59	-3.678	8	0.006*
Limestone (%)	78.85	30.94	370.43	457.30	3.723	8	0.006*
Developed land (%)	1.53	0.39	1.41	0.02	2.138	8	0.065
Open Space (%)	7.07	4.42	3.82	0.03	3.012	8	0.017*
Forested land (%)	25.85	53.48	105.47	38.17	-5.155	8	<0.001*
Total agricultural land (%)	64.65	38.41	64.66	38.08	5.788	8	<0.001*
Agriculture: hay and pasture (%)	35.53	27.74	2.72	24.70	3.329	8	0.010*
Agriculture: cultivated crops (%)	29.12	10.67	58.01	4.68	5.209	8	<0.001*

* significant at p=0.05
† TNC15 excluded as an outlier

Table 4. Mean percentile calculations for total and interior sediment distributions.

	Total Mean Sediment Size (φ)		Interior Mean Sediment Size (φ)	
Percentile	Salem*	South	Salem*	South
D5	8.0	8.0	4.8	3.9
D16	5.7	5.3	1.4	0.8
D25	2.8	0.6	-0.6	-1.8
D50	-3.2	-3.2	-3.7	-3.9
D75	-4.9	-4.8	-4.9	-4.9
D84	-5.8	-5.3	-6.1	-5.6

*TNC15 excluded as an outlier

Table 5. Multiple linear regressions testing the effects of various streambed and watershed variables on macroinvertebrate parameters.

Dependent	Independent	R^2 or Coefficient	Std. Error	p	df	F
EPT/C	(Regression)	0.771	1.977	0.006	2	11.755
	(Constant)	-13.230	4.624	0.024		
	Watershed (Salem=0, South=1)	7.375	1.553	0.002		
	DO (mg/L)	2.097	0.582	0.009		
EPT/C	(Regression)	0.964	0.929	0.001	4	33.335
	(Constant)	-102.937	11.600	<0.001		
	Watershed (Salem=0, South=1)	12.250	1.334	<0.001		
	Ln(Total Phosphorus) (mg/L)	-9.700	1.417	0.001		
	pH	8.435	1.104	0.001		
	Open Space (%)	4.010	0.583	0.001		
mIBI	(Regression)	0.905	1.724	0.009	4	11.842
	(Constant)	24.063	3.991	0.002		
	DO (mg/L)	2.012	0.499	0.010		
	SpC (µS/cm)	-0.018	0.005	0.012		
	Ln(Total Phosphorus) (mg/L)	-3.824	1.086	0.017		
	% Fines (interior)	-0.214	0.054	0.010		
mIBI	(Regression)	0.766	2.462	0.025	3	6.560
	(Constant)	7.601	6.169	0.264		
	Watershed (Salem=0, South=1)	5.371	1.966	0.034		
	Ln(Total Phosphorus) (mg/L)	-4.103	1.545	0.038		
	DO (mg/L)	2.369	0.727	0.017		
FBI	(Regression)	0.850	0.243	0.001	2	19.870
	(Constant)	3.714	0.203	<0.001		
	Watershed (Salem=0, South=1)	-0.525	0.163	0.015		
	% Fines (interior)	0.030	0.007	0.005		
SDI	(Regression)	0.782	0.125	0.005	2	12.546
	(Constant)	-1.259	0.849	0.182		
	Ordinal Date	0.019	0.004	0.003		
	% Fines (interior)	-0.009	0.004	0.043		

Table 6. Multiple linear regressions testing the effects of various streambed and watershed variables on water chemistry and macroinvertebrate parameters.

Dependent	**Independent**	**R^2 or Coefficient**	**Std. Error**	**p**	**df**	**F**
Total Phosphorus (mg/L)	(Regression)	0.962	0.059	<0.001	3	50.488
	(Constant)	-0.201	0.113	0.126		
	Developed (%)	0.455	0.052	<0.001		
	Forested (%)	0.009	0.002	0.007		
	Developed (%) * Forested (%)	-0.010	0.003	0.014		
pH	(Regression)	0.920	0.102	0.001	3	22.991
	(Constant)	5.277	0.340	<0.001		
	DO (mg/L)	0.404	0.053	0.003		
	Nitrate (mg/L)	0.953	0.200	<0.001		
	DO (mg/L) * Nitrate (mg/L)	-0.141	0.028	0.002		
DO (mg/L)	(Regression)	0.761	0.841	0.027	3	6.385
	(Constant)	-17.545	8.120	0.074		
	Forested (%)	0.329	0.116	0.030		
	Cultivated crops (%)	0.357	0.125	0.029		
	Limestone (%)	0.080	0.032	0.045		
Turbidity (FNU)	(Regression)	0.837	1.939	0.009	3	10.254
	(Constant)	-4.248	3.167	0.228		
	Developed (%)	-2.312	0.650	0.012		
	% Fines (total)	0.248	0.065	0.009		
	Watershed Area (km^2)	0.021	0.007	0.023		

Table 7. Selected water chemistry, macroinvertebrate, and sedimentation metrics for each Salem and South fork site.

Fork	**Salem**					**South**				
Site Code	TNC 10	TNC 12	TNC 13	TNC 14	TNC 15	TNC 11	TNC 16	TNC 17	TNC 19	TNC 20
Sampling Date	8/1	7/17	7/13	7/7	8/1	7/21	7/13	7/24	8/2	8/3
Ordinal Date	213	198	193	188	213	202	194	205	214	215
Water Chemistry										
DO (mg/L)	8.36	8.48	7.10	8.86	6.19	6.91	5.20	4.66	7.81	6.50
Nitrate (mg/L)	3.0	2.8	7.4	3.2	0.8	1.9	0.5	0.7	0.5	1.4
pH	8.08	7.85	7.78	7.93	7.81	8.06	7.47	7.42	8.43	7.85
Total Phosphorus (mg/L)	0.256	0.256	1	0.371	0.130	0.267	0.257	0.275	0.279	0.143
SpC (µS/cm)	425.8	744.9	456.6	369.8	285.4	374.4	259.7	212.6	184.7	340.8
Turbidity (FNU)	7.4	3.4	0.2	0.0	12.9	4.7	3.9	6.4	5.2	8.7
Macroinvertebrates										
Abundance	63	72	75	65	96	97	49	96	62	76
EPT/C	3.71	3.71	1.32	6.33	0.55	8.60	3.33	3.31	8.67	12.00
Taxa Richness	22	17	14	14	17	17	13	20	19	20
FBI	4.24	3.82	4.60	4.54	4.85	3.29	3.65	3.97	4.23	3.21
mIBI	31	27	19	31	27	31	27	25	29	33
SDI	2.79	2.40	2.11	2.33	2.41	2.52	2.02	2.53	2.59	2.65
Sedimentation										
D50 (φ units)	-3.8	-3.8	-2.5	-2.9	8.0	-4.6	-2.9	-2.9	-2.4	-3.4
% Fines (total)	26.67	21.90	40.00	33.33	60.95	18.10	25.71	23.81	36.19	28.57
% Fines (interior)	14.29	7.94	31.75	20.63	42.86	3.17	22.22	15.87	26.98	12.70
% Fines (banks)	45.24	42.86	52.38	52.38	88.10	40.48	30.95	40.48	50.00	52.38

Table 8. Pearson correlations for various water chemistry and macroinvertebrate variables with watershed and streambed variables. Only correlations significant at the p=0.05 level are shown.

Parameter 1	Parameter 2	R	P-value
SpC (µS/cm)	Siltstone (%)	-0.745	0.013
	Limestone (%)	0.746	0.013
	Forested land (%)	-0.659	0.038
	Hay and pasture land (%)	0.745	0.013
ANC (mg/L)	Watershed area (km^2)	0.641	0.046
	Siltstone (%)	-0.674	0.033
	Limestone (%)	0.671	0.034
	Hay and pasture land (%)	0.639	0.047
DO (mg/L)	Temperature (◦C)	-0.729	0.017
	Siltstone (%)	-0.647	0.043
	Limestone (%)	0.648	0.043
	Cultivated crop land (%)	0.655	0.040
Nitrate (mg/L)	Siltstone (%)	-0.794	0.006
	Limestone (%)	0.795	0.006
	Developed land (%)	0.969	0.000
	Open Space (%)	0.935	0.000
	Forested land (%)	-0.840	0.002
	Cultivated crop land (%)	0.841	0.002
Total Phosphorus (mg/L)	Turbidity (FNU)	-0.657	0.039
	Developed land (%)	0.902	0.000
	Open Space (%)	0.823	0.003
Turbidity (FNU)	Developed land (%)	-0.643	0.045
	Open Space (%)	-0.675	0.032
	Ordinal Date	0.842	0.002
FBI	Watershed (Salem=0, South=1)	-0.705	0.023
Taxa Richness	Ordinal Date	0.833	0.003
SDI	Ordinal Date	0.770	0.009

Table 9. Multiple linear regressions testing the effects of watershed variables on sedimentation.

Dependent	Independent	R^2 or Coefficient	Std. Error	p	df	F
% Fines (interior)	(Regression)	0.852	5.550	0.007	3	11.485
	(Constant)	17.74	10.881	0.154		
	% Fines (banks)	0.443	0.127	0.013		
	Forested (%)	-1.319	0.378	0.013		
	Siltstone (%)	0.726	0.378	0.010		
% Fines (interior)	(Regression)	0.777	8.401	0.039	2	5.327
	(Constant)	-125.607	44.849	0.026		
	Agriculture (%)	1.98	0.617	0.015		
	Siltstone (%)	0.97	0.299	0.014		

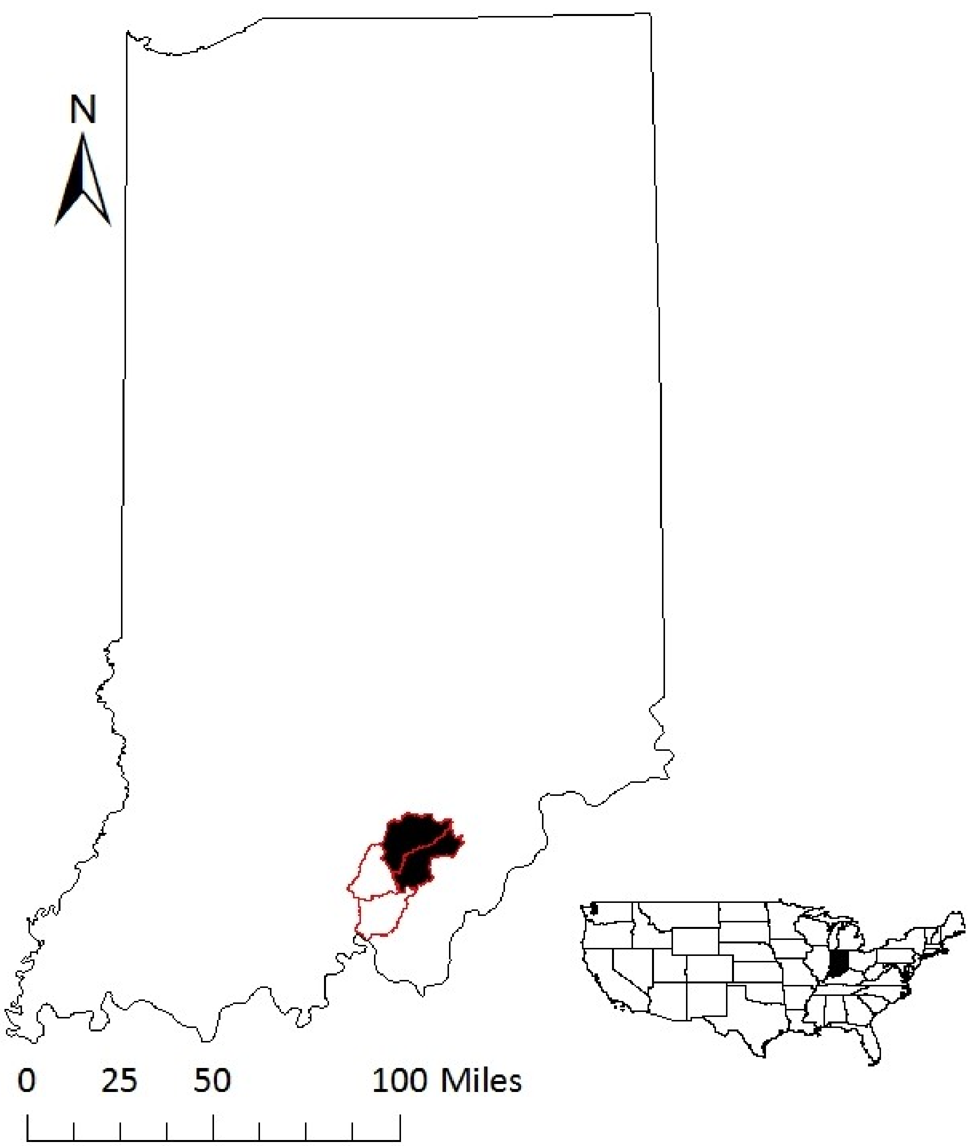


Figure 1. Location of Blue River watershed in Indiana. Blue River sub-watersheds outlined in red, with Salem fork (top) and South fork (bottom) watersheds shown in black (Indiana MAP 2009, USCB 2016).

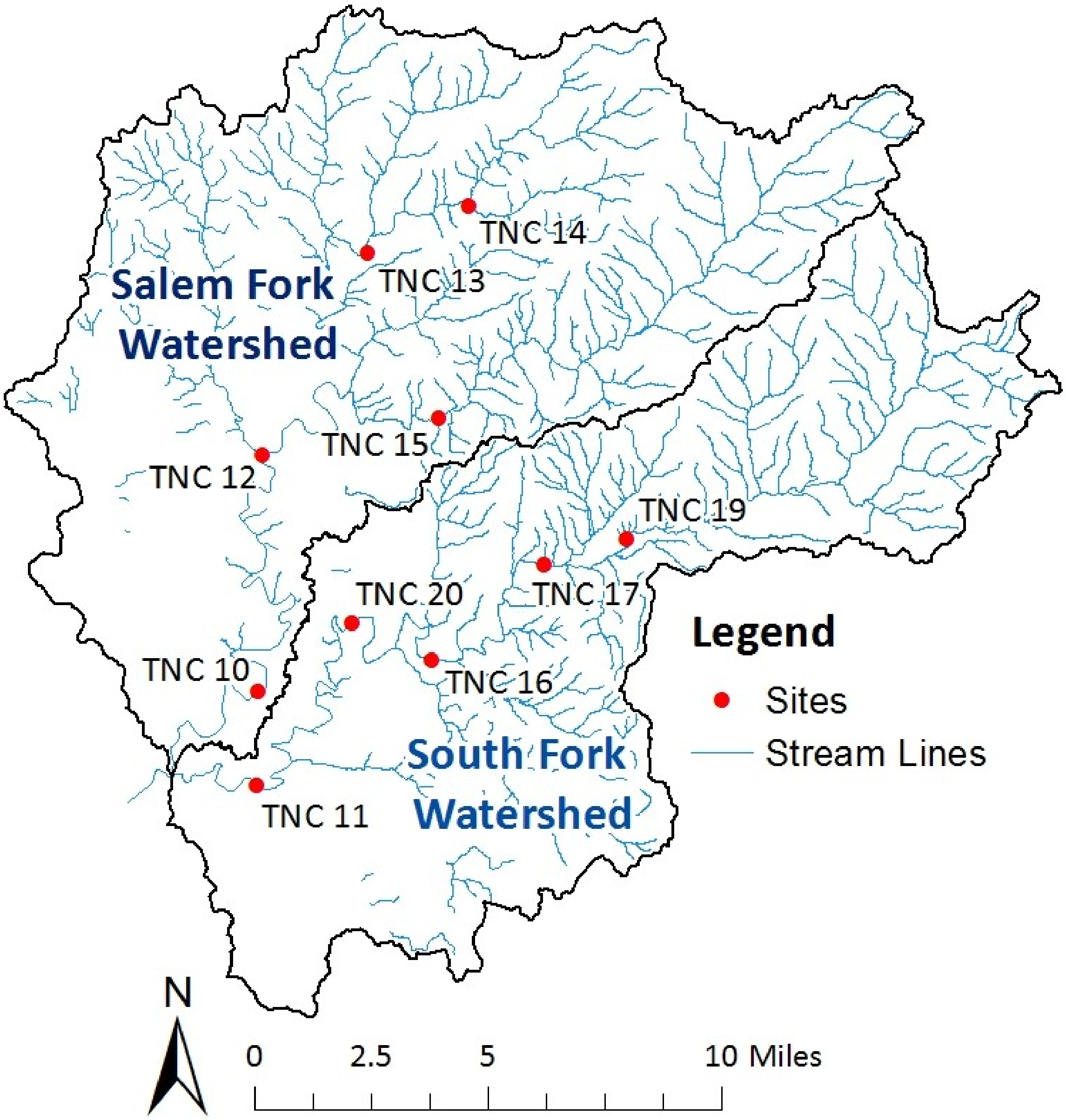


Figure 2. Sites sampled in Salem and South fork watersheds of the upper Blue River during summer 2017 (Indiana MAP 2008, Indiana Map 2009).

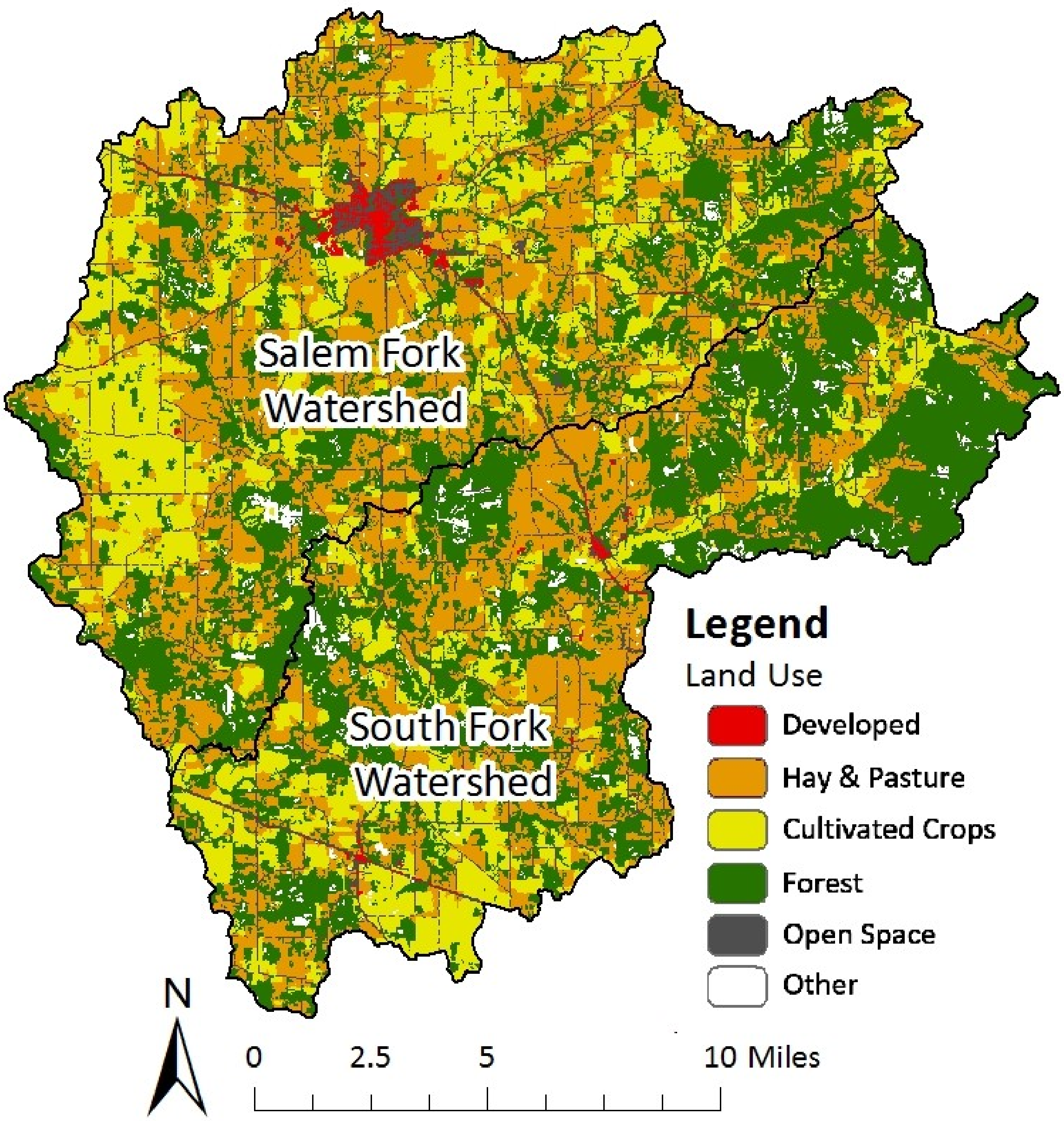


Figure 3. Land use within the upper Blue River's Salem and South fork watersheds (Indiana MAP 2009, Indiana Map 2011).

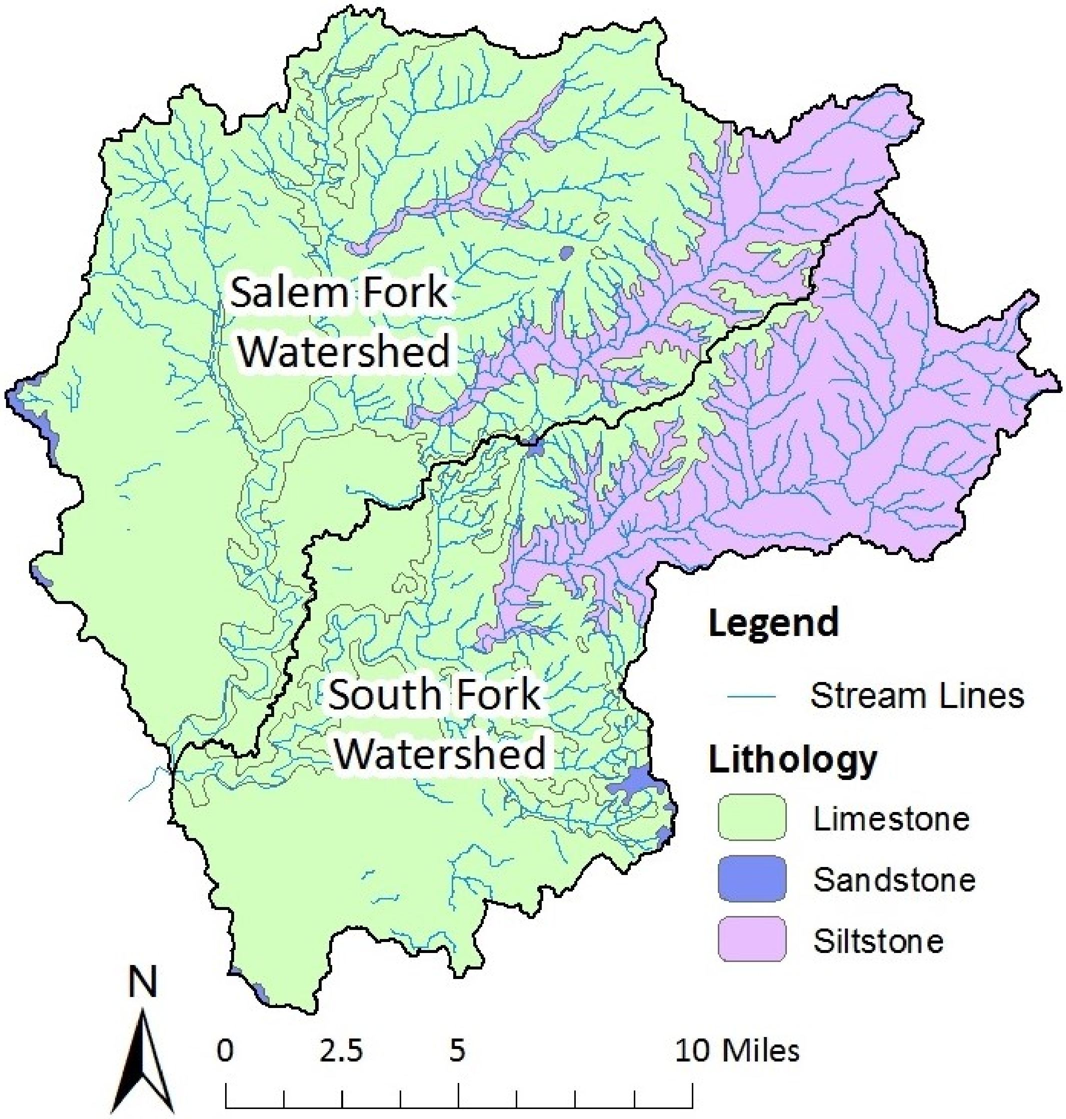


Figure 4. Bedrock geology types within the upper Blue River's Salem and South fork watersheds (Indiana MAP 1987).

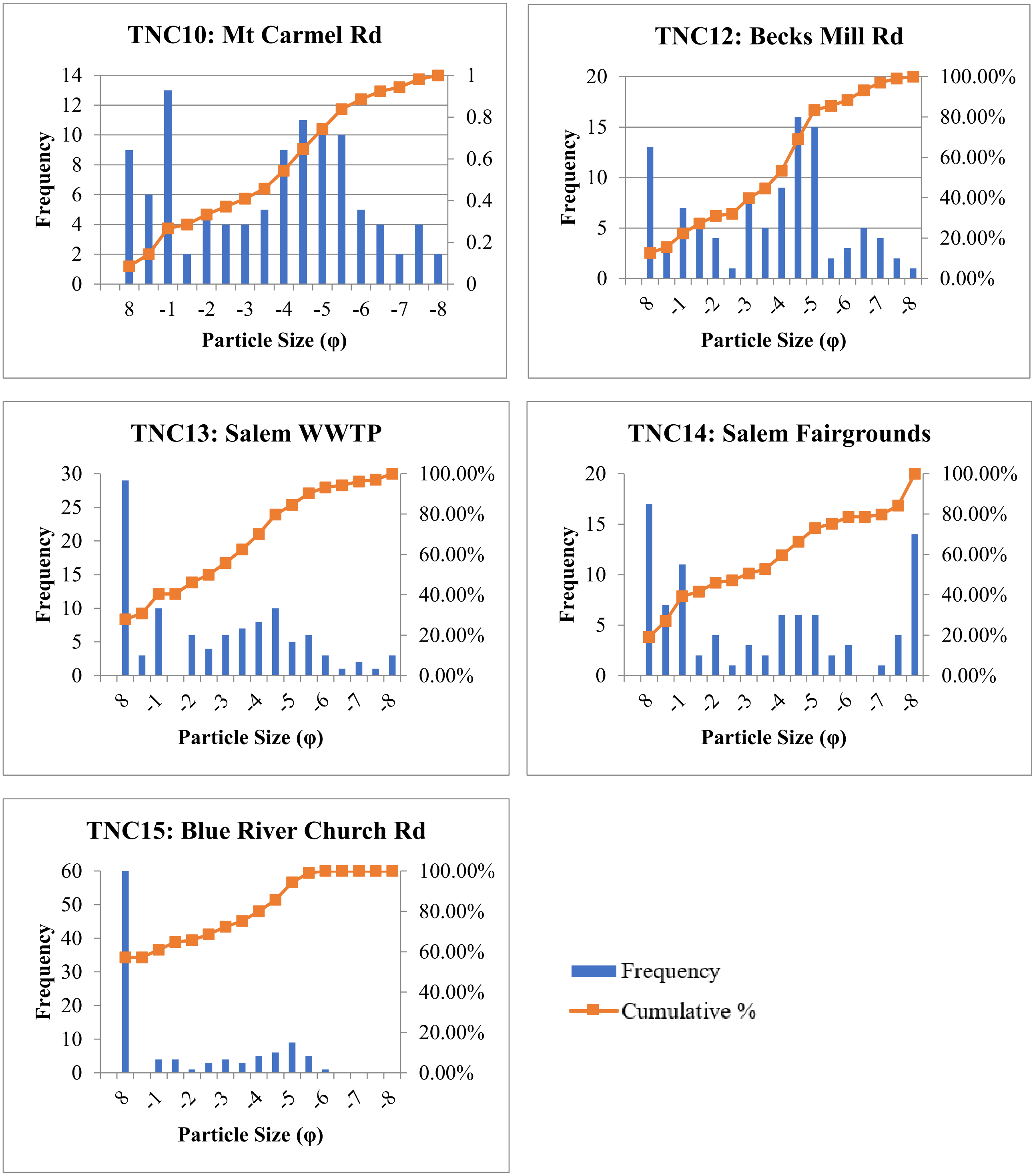


Figure 5. Particle size frequency distributions of substrate at Salem Fork sites.

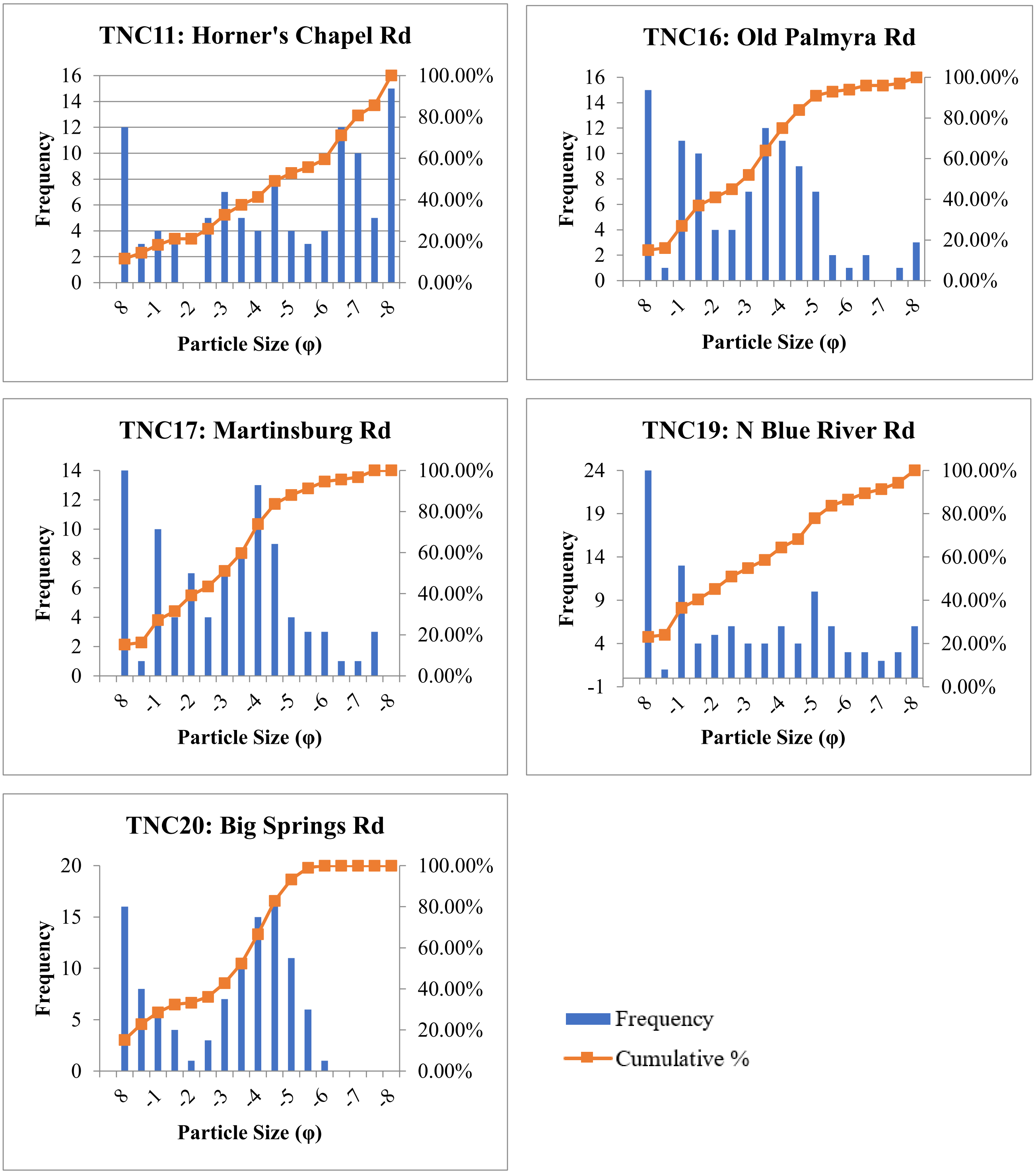


Figure 6. Particle size frequency distributions of substrate at South Fork sites.

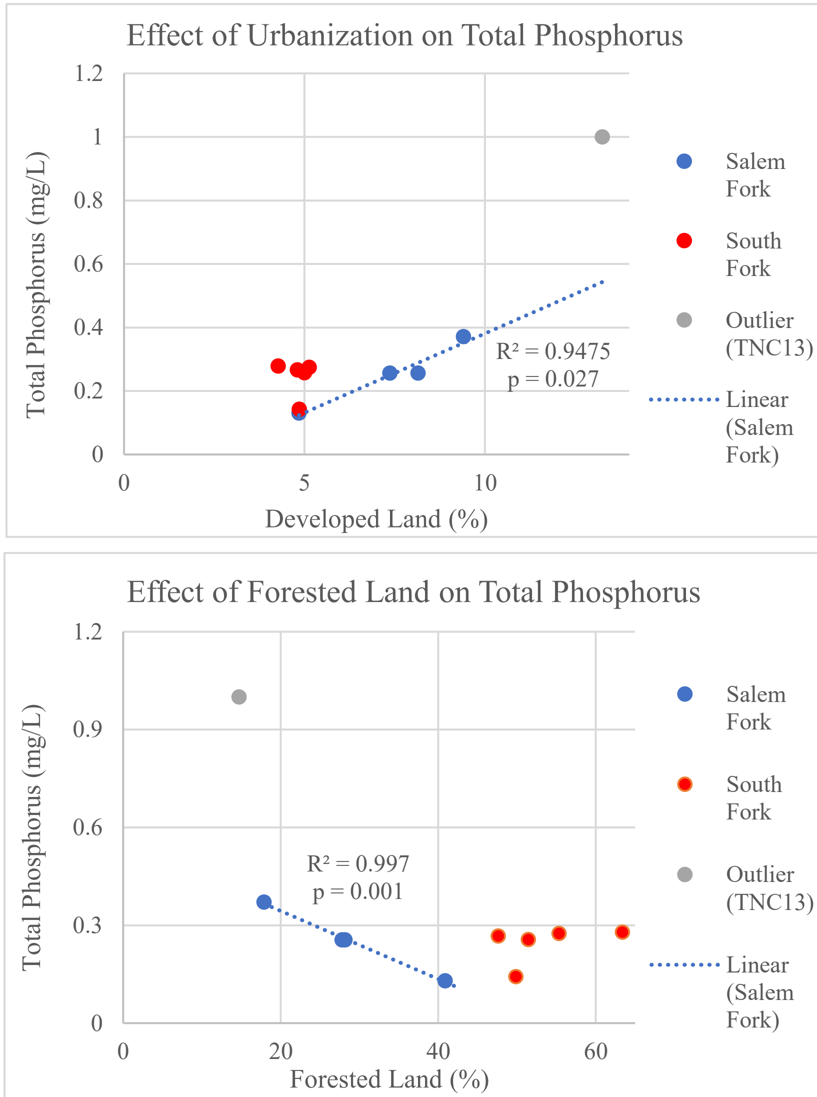
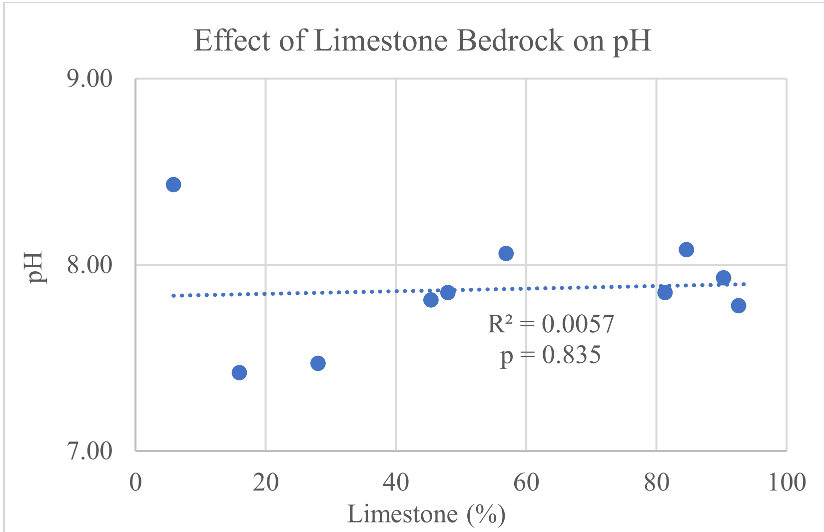


Figure 7. Simple linear regressions of the effects of land use variables on total phosphorus. Significant regressions for Salem fork are shown. TNC13 (Salem WWTP) was excluded as an outlier.



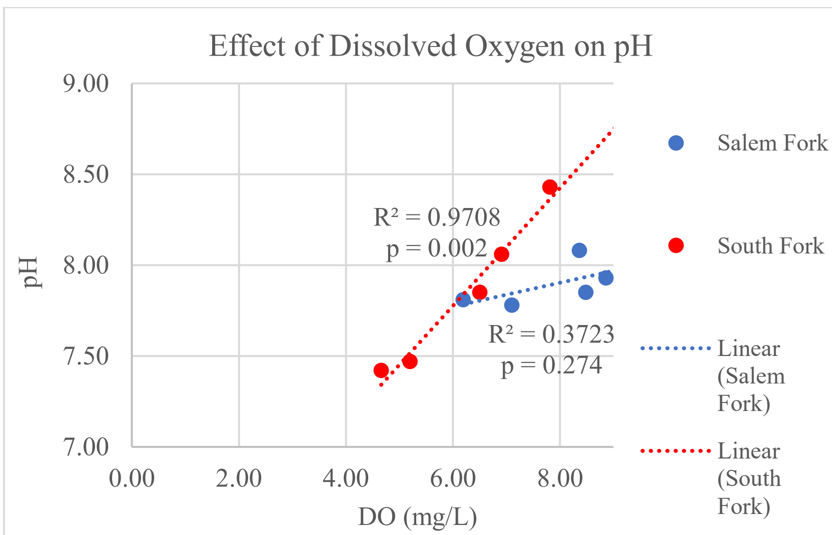


Figure 8. Simple linear regressions showing the effects of limestone and DO on pH.

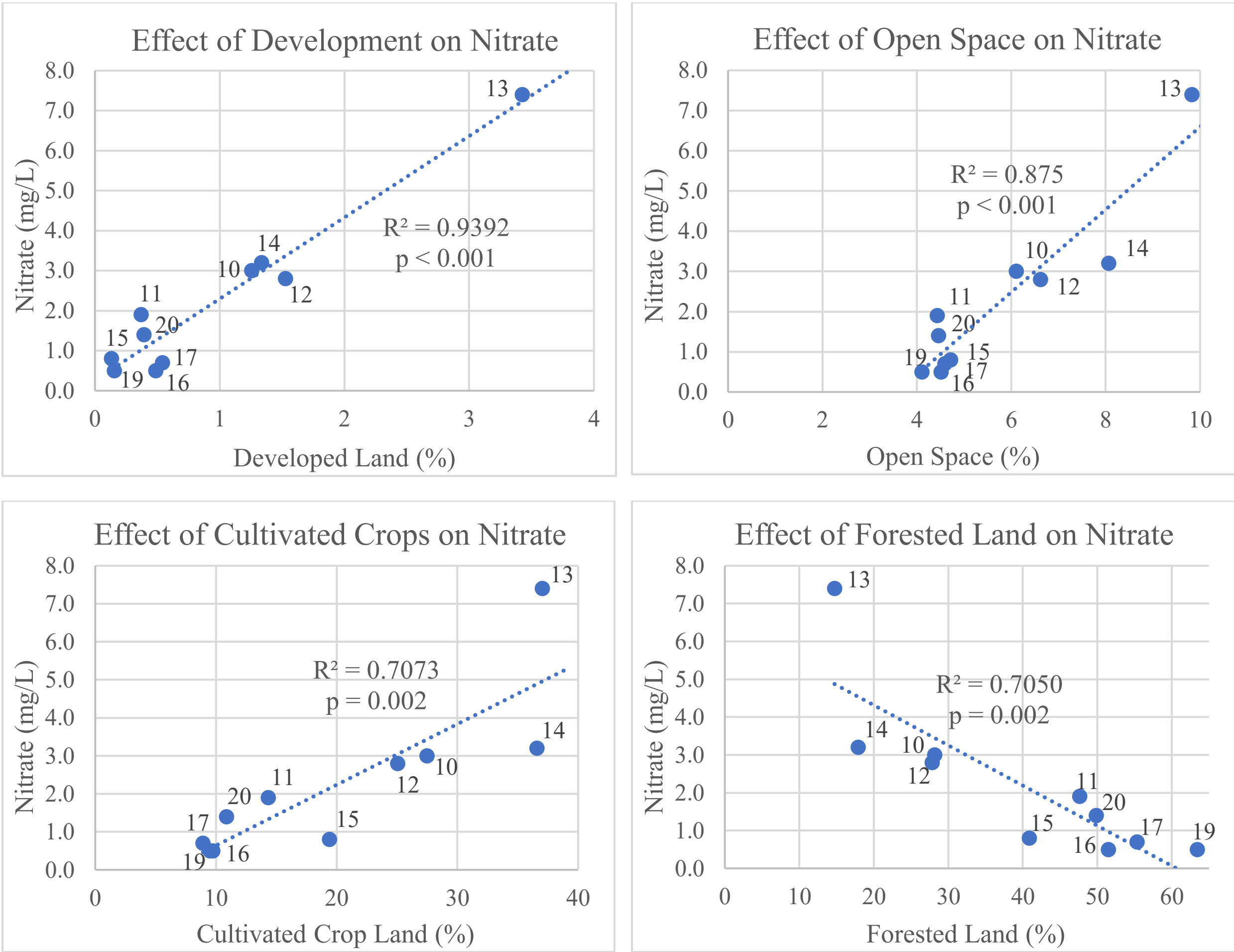


Figure 9. Simple linear regressions of land use variables on nitrate.

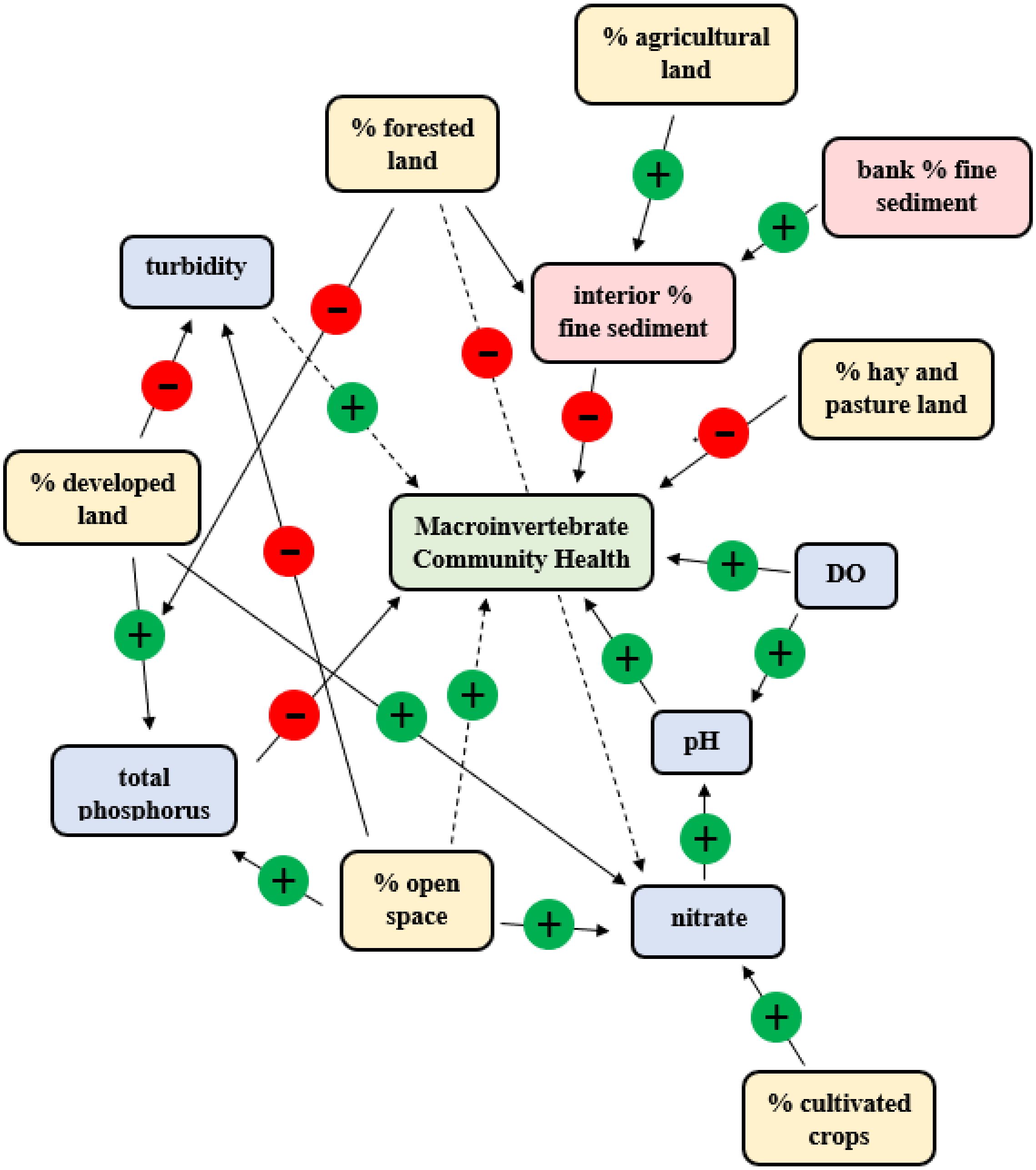


Figure 10. Conceptual model of interactions supported by results of this study.

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